REMARKS

1. In response to the objection on page 2, we have explained the meaning of "Pfl" in claim 6.

2. In response to the indefiniteness rejection of claim 11, we have inserted "all of" as suggested by the Examiner.

Prior Art Issues

1. Claims 6, 7 and 27 are rejected as anticipated by Takahashi or by Yamamoto. These are both new references.

Claims 6, 7, 9-11, 27 and 30 are rejected as anticipated by or obvious over Hugenholtz, Takahashi or Yamamoto in light of the ATCC catalogue. This rejection differs from the one made January 5, 2006.

2. Turning to the merits of the rejection, claim 6 requires a Pfl-defective lactic acid bacterium (which may be a Streptococcus) which meets at least one of five functional conditions (i)-(v), and also satisfies an "obtainable by" process limitation.

The Takahashi et al. reference doesn't in fact teach a Pfl-defective bacterium. Rather, it teaches that a part of the active form of the Pfl of *S. sanguis* can be reversibly converted into an inactive form. That is a post-translational protein modification, not a genetic change. Thus, Takahashi has nothing to do with the presently claimed invention, which relates to Pfl defective classical mutants of lactic acid bacteria.

3. Yamamoto contemplates a Streptococcus mutans mutant (SAK 011) which is Pfl-defective as a result of the genetically engineered deletion of the entire Pfl gene.

The present mutants were discovered by classical microbiological methods,[1] and our specification explains why this

[1] Specifically, treatment with the chemical mutagen ethyl methane sulfonate, P22, L4.

was considered to be advantageous relative to an in vitro genetically engineered microorganism like Yamamoto's. The specification states at P4, L28-30 that

> Accordingly, the prior art does not contain any guidance with respect to designing a feasible method of isolating a lactic acid bacterial Pfl defective (Pfl^-) mutant.

The applicant discovered a viable selection strategy. As stated at P5, L6-17:

> It has now surprisingly been found that wild-type strains of lactic acid bacteria such as strains of *Lactococcus* and *Streptococcus* including as examples *Lactococcus lactis* and *Streptococcus thermophilus* strains under anaerobic conditions grow well on the DN-medium (Dickely et al., 1995) in the absence of acetate. These unexpected findings have made it possible to develop a novel and simple method for the isolation of Pfl defective lactic acid bacterial mutants based on the finding that such mutants, in contrast to the phenotypically Pfl^+ wild-type strains, are unable to grow under anaerobic conditions on DN-medium in the absence of acetate.

At P5, L27-P6, L1 the specification further teaches

> "The above findings have thus opened up for a novel approach for providing useful metabolically engineered lactic acid bacterial starter cultures which approach is based on relatively simple classical random mutagenesis methods or the selection of spontaneously occurring mutants and which does not involve in vitro genetic engineering. From a practical technological point of view this is advantageous, since in most countries the use of genetically engineered food starter cultures is still conditional on approval by regulatory bodies."

The term "mutant" in our claims is discussed at P9, L1-12, which reads (emphasis added):

> "A lactic acid bacterial mutant as defined above can be derived by selecting a spontaneously occurring mutant of a wild-type strain of a lactic acid bacterium... Alternatively, the mutant of the wild-type lactic acid bacterial strain can be provided by subjecting the strain to a mutagenization treatment prior to the selection of a mutant having the above characteristics of the Pfl defective strain".

Said in other words, when our invention refers to "mutant" there is simply meant a spontaneously occurring mutant or mutant obtained from classical random mutagenesis and NOT a genetically engineered plasmid based pfl deletion as described in Yamamoto. We have amended clause (2) of claim 6 to emphasize this.

Yamamoto can anticipate only if Yamamoto's exact organism could satisfy the requirement of the amended claim that the claimed mutant bacterium be "obtainable by a method comprising the steps of

> (1) providing a wild-type lactic acid bacterial strain having a functional Pfl gene, and selected from the group consisting of *Lactococcus* species, *Pediococus* species, *Streptococcus* species and *Bifidobacterium* species, which under aerobic conditions is not capable of growth in the absence of acetate in a medium not containing lipoic acid, but which is capable of growth in such medium under anaerobic conditions, and
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> (2) selecting a mutant, obtainable by spontaneous mutation and/or at least one mutagenization treatment selected from the group consisting of chemical mutagen and ultraviolet light treatment, from said wild-type strain which mutant under said conditions essentially does not grow in the absence of acetate.

Yamamoto of course didn't follow the stated method step (2). However, Yamamoto could still anticipate if Yamamoto's organism could inherently have been "obtainable" by steps (1) and (2).

The general rule concerning inherent anticipation is that the allegedly inherent feature must be certain to be present in view of the explicit features. See Ex parte Levy, 17 USPQ2d 1461, 1464 (BPAI 1990) ("inherent characteristic necessarily flows" from prior art teachings); Glaxo Inc. v. Novopharm Ltd., 29 USPQ2d 1126 (EDNC 1993), aff'd 34 USPQ2d 1565 (Fed. Cir. 1995) (allegedly inherent result must "invariably" happen); Electro Medical Systems, S.A. v. Cooper Life Sciences, Inc., 32 USPQ2d 1017, 1020 (Fed. Cir. 1994) (that a thing "may result" is insufficient); Motorola, Inc. v. Interdigital Technology Corp., 930 F. Supp. 952, 970 (D. Del. 1996); Marion Merrell Dow Inc. v. Geneva Pharmaceuticals, 33 USPQ2d 1673, 1677 (D. Col. 1994); Hughes Aircraft Co. v. United States, 8 USPQ2d 1580, 1583 (Claims Ct. 1988) (in anticipation-by-inherency cases, the element must "flow undeniably and irrefutably from the express disclosures"); Ethyl Molded Products Co. v. Betts Package, Inc., 9 USPQ2d 1001, 1032-3 (E.D. Ky. 1988) (doctrine requires "certainty"; "probabilities are not sufficient"); Phillips Petroleum Co. v. U.S. Steel Corp., 6 USPQ2d 1065, 1076-77 n. 12 (D. Del. 1987), aff'd 9 USPQ2d 1461 (Fed. Cir. 1989) ("anticipation...cannot be predicated on mere conjecture").

We know that the mutation experienced by applicants' strains resulted in inactivation of Pfl. Such inactivation is most likely to be the result of a point mutation in the promoter (interfering with transcription) or in the coding sequence (resulting in production of an inactive protein).

In both spontaneous and chemically induced mutation, deletion of an entire gene is much less likely than a point mutation.

Thus, far from showing that the Yamamoto organism could flow inescapably from the use of a particular classical mutagen, the weight of evidence is that the principal distinguishing feature of that organism -- the inactivation of pfl by complete deletion

-- is very unlikely to have been "obtainable" by the claimed method steps, and no counterexamples has been provided by the Examiner.

Moreover, for Yamamoto to anticipate, it isn't enough that the Yamamoto feature of a completely deleted Pfl be obtainable as claimed; all features of the Yamamoto organism must be so obtainable.

Yamamoto made SAK011 by ligating random Sau3A fragments of S. mutans chromosomal DNA to BamHI-digested pVA891. This plasmid pVA891 is partially derived from E. coli (it is a shuttle vector). The parental strain S. mutans GS-5IS3 was then transformed with the modified pVA891 (pSAK01H2, pSAK1H1B) and it apparently integrated into the parental strain. Yamamoto Fig. 1 compares SAK011, pSAK01H2, SAK1H1B and the parental strain in the Pfl region.

Even setting aside the question of the likelihood of whether deletion of exactly the same piece of the original chromosomal is obtainable by the claimed steps (1) and (2), its replacement with a Sau3A fragment containing **E. coli** DNA is clearly inconceivable to be the result of spontaneous mutation, or chemical- or UV light-induced mutation. Hence Yamamoto doesn't anticipate.

New claim 31 omits recitation of Streptococcus, so it further distinguishes Yamamoto.

5. We now consider the 102/103 rejection of claims 6, 7, 9-11, 27 and 30.

First of all, neither Takahashi nor Hugenholtz disclose any Pfl-defective lactic acid bacteria as claimed. As explained above, Takahashi teaches inactivation of the enzyme (not the gene) by oxygen.

The alleged teachings of Hugenholtz are discussed in the last full paragraph of page 6 of the action and the paragraph

bridging pp. 6-7.[2]

The Examiner says that Hugenholtz' teachings relate to "the group of lactic [acid] bacteria..." This is somewhat ingenuous. The only Pfl-defective lactic acid bacteria discussed by Hugenholtz was of the genus Leuconostoc, as the Examiner admitted on pages 3-4 of the January 5, 2006 office action. Leuconostoc are inherently Pfl defective, see Hugenholtz page 171, col. 2. In contrast, claim 6 as examined recites four other genera: Lactococcus, Pediococcus, Streptococcus and Bifidobacterium.

There is an art-recognized distinction between Genus Leuconostoc and the claimed genera, as was discussed in detail at pages 6-11 of the June 20, 2006 response and the Johansen declaration.

Hence, Hugenholtz doesn't create a reasonable expectation, on the part of those of ordinary skill in the art, that they can by simple selection, or by classical mutation and selection, obtain Pfl-defective mutants of lactic acid bacteria of the four claimed genera.

Nor is this deficiency remedied by Takahashi or Yamamoto. Takahashi doesn't even address mutation, and Yamamoto takes a completely different approach (in vitro genetic engineering), which is disfavored for the reasons set forth in the specification.

Likewise, Hugenholtz doesn't teach how to modify Yamamoto, e.g., specifying point mutations in Streptococcus pfl which would inactivate that gene. Indeed, Hugenholtz merely describes the role of Pfl in citrate metabolism, and notes that it is present in most homofermentative lactic acid bacteria but not in Leuconostoc (page 171, col. 2). Since Leuconostoc don't have Pfl, Hugenholtz could hardly teach how to mutate Leuconostoc Pfl

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[2] There is also a repetition of the page 6 discussion at page 6 lines 3-8.

to inactivate it.

In contrast, the present invention provides a general method of selecting Pfl defective mutants of the claimed genera of lactic acid bacteria.

As we explained in a prior response, there are really several issues here:

(1) Are there in fact an art-recognized taxonomic distinctions between Leuconostoc and the claimed genera?
(2) If such a distinction exists, would the routine worker in the art discount such distinctions when seeking to make a Pfl-defective lactic acid bacterium, if the bacterium in question had a lactic acid metabolism similar to that of Leuconostoc?
(3) Are the lactic acid metabolisms of the bacteria of the claimed genera sufficiently similar to that of Leuconostoc so that the routine worker in the art would find it obvious to apply the teachings of the Leuconostoc references to those claimed genera?

With regard to issue (1), there is no doubt that the art recognizes the existence of a taxonomic distinction between Leuconostoc and the claimed genera. That was true in 1986, as evidenced by Bergey's Manual of Systematic Bacteriology (1986), and it is true today, as evidenced by Appendix 2 to the second (2006) edition, and by the DSM2 "Bacterial Nomenclature Up-to-Date" (March, 2006) (references previously submitted).

There may, of course, be a bit of uncertainty "at the fringe". One organism may be confused with another because of improper examination. For example, Bergey's Manual (1986) states that "gas production from glucose will separate the leuconostocs from the streptococci but this properly should be tested only with actively growing strains, otherwise gas production in the former may not be evident (1073-4). It also says that "normal streptococcal media are unsuitable for leuconostocs and if used can result in misidentification owing to growth".

There can also be organisms which in fact have intermediate

characteristics so their classification is somewhat arbitrary. (Of course, they should still be distinguishable from the more characteristic species of either genus.)

Nonetheless, the utility of the bacterial taxonomy is generally recognized. Indeed, the PTO uses it for patent classification[3], implying its relevancy.

With regard to issue (2), classification of a claimed organism as a different species was given weight in Novo Industri A/S v. Travenol Laboratories, Inc., 211 USPQ 371 (N.D. Ill), opinion supplemented, 211 USPQ 379 (N.D. Ill. 1981), aff'd 215 USPQ 412 (7th Cir. 1982) (Mucor pusillus versus Mucor miehei). Naturally, higher taxonomic distinctions are given even greater weight, see In re Vaeck, 20 USPQ2d 1438 (Fed. Cir. 1991) (Cyanobacteria versus B. megaterium, B. subtilis and E. coli).

The mere mis-identification or reclassification of a single strain of Leuconostoc mesenteroides as Pediococcus is legally inadequate to suggest use of the claimed genera in place of Leuconostoc.

Absent some suggestion, in the prior art and not obtained by hindsight, the routine worker would not "cross taxonomic lines". The difference in taxonomy would lead the routine worker to expect differences in metabolism which would affect the expectation of success.

Turning to issue (3), the art would have been aware of specific differences in lactic acid metabolism between Leuconostoc and the claimed genera. These would tend to defeat any suggestion, or expectation of success, which even arguably might be created by the classification history.

It is true, of course, that a particular strain doesn't change its metabolic pathways. However, that doesn't mean that

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[3] See e.g., 424/93.4-93.48, 435/7.32-7.37, 435/36, 435/221, 435/252.1-253.6, 435/822-910.

there aren't relevant metabolic differences among the lactic acid bacteria, which are formally defined at page 8, lines 23-32.

Fig. 1 and Table 1 in Starrenburg evidence significant differences in lactose and citrate metabolism, respectively, between Lactococcus lactis and two Leuconostoc species.

The lactic acid metabolism of Leuconostoc is further compared to that of the claimed genera Lactococcus, Streptococcus, Pediococcus and Bifidobacterium in the previously submitted Declaration of Eric Johansen.

The distinctions are summarized in the table below:

Leuconostoc	heterofermentative; CO_2 produced
Lactococcus Streptococcus	homofermentative
Pediococcus	homofermentative; no CO_2 production
Bifidobacterium	heterofermentative; CO_2 produced only from gluconate

The Examiner asserts

> Some of the arguments and the contents of the declaration are directed to the idea that lactic acid metabolic pathways are distinct in the homofermentative lactic acid bacteria and in the heterofermentative lactic acid bacteria. However, as related to the pyruvate metabolism and the pfl inactivation the lactic bacteria are the same and/or similar since lactic bacteria convert sugars to lactic acid via pyruvate as intermediate product. Although the exact amounts of specific end products in homofermentative and heterofermentative lactic acid bacteria might be different, the inactivation of pfl in lactic acid bacteria result in the presently features including "essentially no production" of formate, acetate and ethanol as adequately taught and/or suggested by Hugenholtz et al. and Takahashi et al.

The Examiner's own reference, Hugenholtz, states that "in

Leuconostoc spp. isolated from dairy sources pyruvate metabolism is (much) less complex than in lactococci" (p. 169, col. 1). This contradicts the examiner's assertion that they are "the same or similar". As to Pfl inactivation, Hugenholtz provides no guidance as to how this is to be accomplished.

The ATCC catalogue pages are perfunctorily cited again as demonstrating that "the bacterial species of lactic [acid] bacteria have been frequently cross-identified and reclassified between these genera and species". The word "frequently" is rather clearly abused here. The Examiner cited five ATCC pages.

ATCC Strain	ATCC page	Original ID	Current ID
15520	205	Lactobacillus batatas	Leuconostoc lactis
8042	264	Leuconostoc mesenteroides	Pediococcus acidilacti
11863	68	Lactobacillus bifidus	Bifidobacterium bifidum
7962	199	Streptococcus lactis	Lactococcus lactis
11007	199	Streptococcus diacetilactis	Lactococcus lactis

We think it likely that the correction evidenced by ATCC page 205 was prompted by a reclassification (since L. batatas is no longer on the list of valid bacterial species names), but it is irrelevant whether genus Lactobacillus is distinguishable from genus Leuconostoc (they are both are recognized by the art as separate genera) since we don't claim either genus.

Turning to Pediococcus, the Examiner relies on ATCC 8042, initially identified as Leuconostoc mesenteroides, and now listed as Pediococcus acidilactici.

In Pediococci, glucose is fermented to DL or L-(+)-lactate (Bergey's, p. 1075). Again, gas is not formed. This clearly distinguishes Leuconostoc in the critical area of lactic acid

metabolism.

Bergey's also comments that pediococci are morphologically distinct from other lactic acid bacteria.

It is not clear to us why ATCC 8042 was first identified as Leuconostoc mesenteroides (Bergey's p. 1074) rather than Pediococcus acidilactici (Bergey's 1079) but we think it inappropriate to infer from a single mistake that the Leuconostoc and Pediococcus genera can be lumped together, given the relevant metabolic and morphologic differences.

With respect to ATCC 11863, it is quite clear that Bergey's Manual recognizes the Lactobacilli and the Bifidobacteria as being "completely unrelated" and so no person of ordinary skill in the art would extrapolate from Lactobacilli to Bifidobacteria.

The remaining citations (ATCC 7962, 11007) relate to the relationship of the Streptococci to the Lactococci. This relationship is of course of no import whatsoever insofar as Hugenholtz's Leuconostoc are concerned.

Yamamoto genetically engineered a Streptococcus mutans. This is not one of the Streptococcal species for which the ATCC catalogue evidences misidentification or reclassification relative to a lactococcal species.

However, even if it were reasonable to lump together the Streptococci and Lactococci, the fact that one of Yamamoto's ten thousand random streptococcus mutans transformants was Pfl-defective as a result of replacement of the native pfl gene with a non-Pfl Sau3A fragment doesn't make it any easier for the art to obtain a Lactococcus mutant by means other than in vitro genetic engineering. In particular, none of the cited art teaches inactivating point mutations to incorporate into pfl, let alone the mutations characterizing our DN221 and DN227, or other pfl mutation likely to result from EMS treatment (cp. claim 34).

6. The Examiner concedes that claim 11 (drawn to the DN221 and DN227 isolates) "might be allowable upon resolution of the

112-2 issues(s)", see page 9, third full paragraph. It is unclear to what extent this concession is tempered by the discussion of claim 11 in the last full paragraph on page 8.

We are sure the Examiner will appreciate that we would need stronger assurance than "might be allowable" in order to consider limiting the claimed subject matter to claim 11.

In the interest of expediting prosecution, we have presented new claims 35-38, which are intermediate in scope between claims 10 (Lactococcus lactis subspecies lactis) and 11 (DN221 and DN227). These cover mutants derived, by specified techniques, from DN221 and DN227 which retain the characteristics specified by claim 6. In Ex parte Jackson, 217 USPQ 804 (BPAI 1982), claim 3, reciting deposited strains and mutants thereof, was held enabled even though certain broader claims weren't.

Respectfully submitted,

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